Coenzyme \( Q_{10} \) Exogenous Administration Attenuates Cold Stress Cardiac Injury

Neif MURAD,\(^1\) MD, Katia TAKIUCHI,\(^1\) MD, Antonio C. LOPES,\(^1\) MD, André M. M. BONILHA,\(^1\) MD, Marcia M. SOUZA,\(^1\) MD, Léa Maria M. F. DEMARCHI,\(^2\) MD, Maria De Lourdes HIGUCHI,\(^2\) MD, and Paulo J. F. TUCCI,\(^1\) MD

**SUMMARY**

The influence of coenzyme \( Q_{10} \) (CoQ\(_{10}\)) in cold stress test (-15°C for 4 hours) cardiac functional impairment was studied in isolated isovolumic heart of control rats (C; \( n=12 \)) and of placebo (P; \( n=11 \)) and treated rats (CoQ\(_{10}\); \( n=10 \)). In addition, electron microscopic evaluation of left ventricular (LV) slices (\( n=3 \) in each group) allowed us to analyze the myocardial ultrastructure. Maximal values of developed pressure (DP\(_{max}\)) were similarly decreased in cold stressed animals (C=129±3.9 mmHg; P=106±6.7 mmHg; CoQ\(_{10}=91±3.9 \) mmHg); however, volume-induced enhancement of pressure generation (slope of DP / volume relations: C=0.248±0.0203 mmHg / µl; P=0.2831±0.0187 mmHg / µl; CoQ\(_{10}=0.2387 \) (0.0225 mmHg / µl; \( p>0.05 \)), and the duration of systole (C=80±1.6 ms; P=78±1.3 ms; CoQ\(_{10}=80±2.7 \) ms) were not altered. Myocardial relaxation, evaluated by the relaxation constant (C=39±1.9 ms; P=42±3.4 ms; CoQ\(_{10}=51±6.0 \) ms), as well as resting stress / strain relations were unaffected by cold stress. Myocardial samples showed that pretreatment with CoQ\(_{10}\) attenuates myofibrillar and mitochondrial lesions, and prevents mitochondrial fractional area increase (P: 53.11%>CoQ\(_{10}\): 38.78%=C: 33.87%; \( p<0.005 \)) indicating that the exogenous administration of CoQ\(_{10}\) can reduce cold stress myocardial injury. (Jpn Heart J 2001; 42: 327-338)

**Key words:** Ubiquinone, Coenzyme \( Q_{10} \), Cold stress test, Systolic function, Diastolic function

**CONSOLIDATED data from the literature point towards the existence of myocardial structural lesions resulting from the exposure of animals to extreme low temperatures.**

More frequently, the structural myocardial lesion described is characterized by vacuolization of mitochondrial cristae and myofilament disarrangement.\(^{1-4} \) The sharp increase of circulating cathecolamines is likely to be a factor in myocardial lesions.\(^{5-8} \) It has been demonstrated that previous administration of propanolol prevents cold stress test induced structural changes.\(^4\) To our knowledge, there is only one paper describing the sole influence of profound sys-
temic cold stress on ventricular function; decreases of contraction amplitude, and myocardial stiffness of isolated rat heart. Other papers analyzing the action of hypothermia over cardiac function attempted to assess myocardial preservation in a surgical setting and in these studies hypothermia was always associated with ischemia and reperfusion injury.

Coenzyme Q_{10} (CoQ_{10}) is an enzyme naturally synthesized by the organism as a mobile electron carrier in the mitochondrial electron-transfer process of respiration and coupled phosphorylation. CoQ_{10} has been shown to prevent cardiac injury resulting from sustained tachycardia, adriamycin toxicity and ischemia / reperfusion. It has been demonstrated that the exogenous administration of CoQ_{10} blocks lipid peroxidation, reduces depletion of ATP, improves high-energy phosphate restoration of postischemic reperfusion, and reduces mitochondrial calcium overload due to ischemia reperfusion. Its ability to scavenge oxygen free radicals has also been suggested. Ultrastructurally, CoQ_{10} has been shown to prevent mitochondrial deformity during episodes of ischemia. Nayler and al demonstrated that pretreatment of animals with CoQ_{10} was able to maintain mitochondrial architecture, compared with nontreated animals, which showed edema, cell membrane disruption, mitochondrial lysis, and disorganization of myofibrils during postischemic reperfusion. To our knowledge, no report has analyzed the action of CoQ_{10} action on myocardial structural and functional lesions resulting from cold stress test.

The purpose of this paper was to analyze the action of myocardial injury resulting from cold stress test on cardiac mechanics as well as the modifications caused by CoQ_{10} on functional and ultrastructural changes. In the clinical setting, the results may lead to an understanding of whether the exogenous administration of CoQ_{10} protects the myocardium from injuries. In isolated isovolumic rat hearts systolic and diastolic function parameters were analyzed and the ultrastructural aspects were assessed by electron microscopy.

**Materials and Methods**

**Animals:** The study was conducted on 42 male Wistar rats weighing 250-300 g. Thirty-three animals were used for functional data assessment and 9 for the ultrastructural parameters. Rats were matched in one of the three experimental groupings: a) CONTROL (C; n=15): those in this group received 1 ml of Ubiquinone vehicle (carboxymethyl cellulose+methylparaben+propyl paraben), by gastric gavage, for five days prior to the cold stress test. Twelve rats were used for functional data definition, and three for ultrastructure analysis; b) PLACEBO (P; n=14): those rats received 1 ml of vehicle, by gastric gavage, for five days prior to an experimental procedure, and were kept in the refrigerator for four hours at
COENZYME Q10 AND COLD STRESS TEST

-15°C. Eleven rats were used in the functional studies and three in the ultrastructure study; c) COENZYME Q_{10} (CoQ_{10}; n=13): these rats received 1 ml of solution which contained 10 mg of CoQ_{10} (kindly supplied by Marjan Farma Ltda, São Paulo, Brazil) for five days preceding the experiment and were kept in the refrigerator for four hours at regulated -15°C. Ten animals were studied in the functional assessment experiments, and three in the ultrastructure analysis.

Immediately after the cold stress test the animals were used in the functional and ultrastructure evaluations. After intraperitoneal heparinization (500 IU) and anesthesia (urethane: 1.2 g / kg), the animals underwent a medial thoracotomy, the ascending aorta was cannulated and the heart perfused at a constant perfusion pressure of 100 mmHg using the Langendorff technique in a retrograde manner, with Krebs-Henseleit bicarbonate buffer, pH 7.35-7.45, containing 10 U / l insulin bubbled with 95% O₂ and 5% CO₂ (P'O₂>480 mmHg) and warmed to 36°C. The heart was then quickly removed, the LV cavity vented by apical puncture and a small collapsed latex balloon was placed inside the LV chamber via the left atrium. The balloon was connected to a pressure transducer by a short length of stiff polyethylene tubing to determine LV pressure. The balloon was sufficiently compliant and its size large enough so as not to contribute to pressure over the volume range utilized (maximum volume introduced in the balloon: 400 µl). The right atrium, including the sinus node region, was removed and right ventricular pacing was carried out using a right ventricular bipolar pacemaker wire driven by a stimulator delivering 4 ms monophasic square waves of 5 V. A stimulation rate of 180 beats / minute was used in all experiments. When needed, atrioventricular (AV) block was effected by mechanical crushing of the AV nodal region to control heart rate. The LV volume was adjusted in such a way that diastolic pressure was zero (V₀).

After the equilibration period (15 min), a pressure-volume relation was determined for every heart by measuring LV peak systolic and diastolic pressures 30 s after 20 µl increments in the balloon volume (saline injected with a 500 µl Hamilton syringe) until a maximum end diastolic pressure of 40 mmHg was obtained. An electronic derivative of the LV pressure (dP / dt) was also recorded.

At the end of the experiments, the left atrium and right ventricular free wall were removed and the myocardial mass was weighed and taken as the LV wet weight.

Nine other rats, whose hearts were not perfused, were utilized for ultrastructural analyzes. Immediately after anesthesia, the thorax of each rat was opened and the heart was exposed and removed. Cold 2% glutaraldehyde solution was dropped on the still beating heart. Fragments of the left ventricle wall were then removed and fixed in phosphate buffered glutaraldehyde at pH 7.2. The fragments were cut into small, 1 mm thick pieces, which were postfixied in 1% OsO₄.
solution for 2 h at 4°C, and then dehydrated and embedded in araldite. Silver or gray thin sections were cut on a Porter-Blum MT-B ultramicrotome, mounted on copper grids and stained with uranyl acetate and lead citrate. The preparations were examined through a Philips EM-301 electron microscope and photographed at 2,000x the original magnification.

A reticule with 1,750 points was superimposed to three representative microphotographies (14,380X - final magnification) of each rat and the fractional mitochondrial area was calculated according to the method described by Gundersen, et al. The mean of the three measures was considered as representative of each rat.

Calculations: Developed pressure (DP: systolic pressure minus diastolic pressure) was analyzed and maximal developed pressure (DP\textsubscript{max}) was utilized as an index of maximal contractile capacity. Frank-Starling curves were constructed for each heart plotting instantaneous developed pressure as a function of the respective left ventricular volume. The points of the ascending limb of pressure/volume relations, which maintain a linear relation, were linearly fitting and the slopes of the straight lines were compared. Systole duration was defined in the pressure record by the time to peak pressure (TPP: time elapsed from the beginning of contraction until maximal value of DP).

Myocardial stiffness was evaluated in all cases. Resting circumferential wall stress was estimated from ventricular pressure measurements, ventricular volume and weight as described by Bing, et al. A spherical model was assumed, in which the left ventricular cavity has a radius R\textsubscript{i}, and chamber volume (V\textsubscript{c}) was considered to be the sum of the liquid volume inside the balloon plus the latex volume of the balloon (latex weight / 0.936, the specific gravity of latex balloon\textsuperscript{19}). Thus, V\textsubscript{c}=\(\frac{4}{3} \pi R_{i}^{3}\), and R\textsubscript{i}=[V\textsubscript{c} / (\frac{4}{3} \pi)]\textsuperscript{1/3}. Total heart volume (contained in a sphere of radius R\textsubscript{i}+h) is equal to the sum of V\textsubscript{c} and V\textsubscript{wall}, where V\textsubscript{wall} is the volume of the LV\textsubscript{wall} (V\textsubscript{wall}=left ventricular net weight / 1.05, the specific gravity of the myocardium). Therefore, V\textsubscript{c}+V\textsubscript{wall}=\(\frac{4}{3} \pi (R_{i}+h)^{1/3}\), and R\textsubscript{i}+h=[vc+V\textsubscript{wall} / (4/3 \pi)]\textsuperscript{1/3}. LV diastolic mid-wall circumferential stress (\(\sigma\)) was then derived from the relationship described by Mirsky\textsuperscript{25}:

\[
\sigma=(PR_{i}^{2}/h) / (2R_{i}+h)
\]

where P is resting pressure, R\textsubscript{i} is chamber radius and h is wall thickness.

Passive myocardial stiffness curves were established by fitting the resting stress (\(\sigma_{r}\))-strain (\(\varepsilon\)) data to a mono-exponential relation \(\sigma_{r}=A \left(e^{K\varepsilon} -1\right)\) where A and K are coefficients.

In order to evaluate myocardial relaxation, the time constant for isovolumic pressure decline (\(\tau\)) was calculated according to the equation P\textsubscript{t}=P\textsubscript{0}e\textsuperscript{-\(t/\tau\)}, where P\textsubscript{t} is LV pressure at time t, and P\textsubscript{0} is LV pressure at the peak rate of LV pressure fall (nadir of dP / dt). Pressure measurements were taken at 10 ms intervals at a paper
speed of 100 mm / s and linear regression analysis was performed for the plot of \( \ln P_t \) vs \( t \). Subsequently, \( \tau \) was defined as the negative reciprocal of the slope, according to the equation \( \ln P_t = -t / \tau + \ln P_0 \).

**Statistical analysis:** One way ANOVA complemented by the Newman-Keuls test was used to detect differences among groups. A \( p \) value<0.05 was considered significant and the results are presented as means±standard error of the means (x±SEM).

**RESULTS**

No differences could be seen between myocardial wet weight (C= 627±8.9 mg; P=619±9.9 mg; CoQ10=649±13 mg; \( p > 0.05 \)) and ventricular volume at zero diastolic pressure (C=122±9.5 \( \mu l \); P=106±7.9 \( \mu l \); CoQ10=112±5.9 \( \mu l \); \( p > 0.05 \)) for the three groups.

**Functional data:** Similar impairment of systolic function was noted for groups P and CoQ10, since their \( DP_{\text{max}} \) (P: 106±6.7 mmHg; CoQ10: 91±3.9 mmHg; Figure 1A) and \( dP / dt \) (P: 2,086±123 mmHg / s; CoQ10: 1,994±99 mmHg / s; Figure 1B) did not differ and were significantly lower (\( p < 0.001 \)) then control hearts (129±3.9 mmHg and 2,482±80 mmHg / s, respectively). Developed pressures / ventricular volume relations have shown that myocardial sensitivity to stretching has been preserved in animals submitted to cold stress test (Figure 1C). Although the P and CoQ10 groups straight lines are seen to be under that of controls, no signif-

![Figure 1](image_url)

**Figure 1.** **Panel A:** Individual values (empty symbols), means (filled symbols) and standard error of means (vertical bars) of maximal developed pressure (\( DP_{\text{max}} \)) of hearts of the Control (triangles), Placebo (inverted triangles) and CoQ10 (circles) groups. **Panel B:** Individual values (empty symbols), means (filled symbols) and standard error of means (vertical bars) of \( dP / dt \) of the Control (triangles), Placebo (inverted triangles) and CoQ10 (circles) groups. **Panel C:** Developed pressure (DP) plotted as a function of ventricular volume of hearts of the Control (triangles), Placebo (inverted triangles) and CoQ10 (circles) groups.
icant difference between the slopes could be identified in the three groups (C: 0.248±0.02 mmHg / µl; P=0.283±0.018 mmHg / µl; CoQ_{10}=0.238±0.022 mmHg / µl; p>0.05). These results indicate that rats submitted to cold stress test had their contractile capacity decreased all along the Frank-Starling curve, however, the myocardial sensitivity to stretching was preserved. In addition, no changes were observed in contraction duration (TPP) for animals submitted to cold stress test (C=80±1.6 ms; P=78±1.3 ms; CoQ_{10}=80 (2.7 ms; p>0.05).

Diastolic function analyses showed that cold stress did not affect myocardial relaxation or stiffness. The stress / strain curves for the three groups were superimposed (Figure 2A), making the myocardial stiffness calculation (dσ / dε) dispensable. Likewise, no significant differences were identified between the relaxation constant in the three groups (C: 39±1.9 s; P: 42±3.4 s; CoQ_{10}: 51±6.0 s; p>0.05; Figure 2B).

Figure 2. A: Resting stress (RS)/strain (V-V₀/V₀) relationships of hearts of Control (triangles), Placebo (inverted triangles) and CoQ_{10} (circles) groups. B: Individual values (empty symbols), means (filled symbols) and standard error of means (vertical bars) of relaxation constant (τ) of hearts of the control (triangles), placebo (inverted triangles) and CoQ_{10} (circles) groups.
Ultrastructure data: The myocardium of the C group animals showed a normal morphology which consisted of elongated striated muscle cells with preserved myofibrils and sarcolemma. Interstitial connective tissue components, such as fibroblasts, collagen fibrils, amorphous intercellular matrix and blood vessels were observed. The sarcomeres in the sarcoplasm were organized and delimited by Z bands, and showed slightly fainted I bands and, in some cases, sarcoplasmic reticulum dilatation. Between the myofibrils or in the sarcoplasm, there was a high concentration of mitochondria of several sizes and shapes with no structural changes. Few lipid droplets were also observed in the sarcoplasm.

The P group cardiac fibers (Figure 3A and 3B) presented areas in which the myofibrils were well preserved, with a structural pattern similar to normal, and numerous areas with focal loss of myofibrils. Relaxation of the sarcomeres was also observed, characterized by enlargement of I bands. A great number of mitochondria showed alterations of different intensity such as swelling, a decrease in number and disruption of the cristae with formation of large vesicles. Amorphous dense substance was not observed.

Left ventricle cardiac fibers from animals protected by CoQ10 showed (Figure 3C) few foci of myocardial fibers with structural changes constituted by rare areas of loss of the myofilaments, mitochondrial swelling, with a decrease in the

![Figure 3](image-url)

Figure 3. Electron micrographs (14,380X final magnification) of left ventricular myocardium of placebo (Panel A and B) and rats treated with CoQ10 (Panel C) showing focal loss of myofibrils (∗), mitochondrial swelling (●), mitochondrial swelling and cristolysis (●), clear I band enlargement (●) and sarcoplasmic reticulum dilatation (▲).
number of cristae and absence of amorphous dense deposits. Overall, the sarcomeres showed narrow I bands and rare lipid droplets.

Morphometric analysis of the mitochondrial fraction area evaluated the organelle damage in the P group and the protection offered by CoQ10 (Table). Indeed, the mitochondrial fraction area of the animals from the CoQ10 group (38.8 ± 0.2%) did not differ from that of the control group animals (33.9 ± 0.9%) and both were smaller (p < 0.005) than that observed in placebo animals (53.2 ± 4.5%)

<table>
<thead>
<tr>
<th>Rat</th>
<th>Control</th>
<th>Placebo</th>
<th>Co Q₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.29</td>
<td>57.74</td>
<td>38.91</td>
</tr>
<tr>
<td>2</td>
<td>35.56</td>
<td>57.48</td>
<td>38.35</td>
</tr>
<tr>
<td>3</td>
<td>33.75</td>
<td>44.11</td>
<td>39.07</td>
</tr>
</tbody>
</table>

x ± SEM: 33.87 ± 0.95 53.11 ± 4.5 * 38.78 ± 0.22

* = p<0.05 from control. § = p<0.05 from Placebo.

DISCUSSION

Most common literature references to profound cold cardiac effects are related to experimental studies in the surgical setting. In this scenario, the cardiac effects of profound cold temperature are evaluated in isolated hearts, in association with ischemia / reperfusion and this peculiarity hinders a comparison with our data. In addition, the reference points of temperature utilized for cold stress test in physiological analyses conducted in intact organisms are usually markedly higher than those achieved in the present experiments, also hampering a consistent comparison with our data. As a rule, studies of cardiac effects consequent to profound cold stress carried out in intact organisms only focus on ultrastructural myocardial lesions. Mitochondrial and myofibrillar lesions, as was noted in the present report, seem to be a remarkable feature in this condition. To our knowledge, there is only one previous report on the functional cardiac consequences of this situation.9) Systolic impairment and myocardial stiffness decreases were described after profound cold stress.

Our data have shown that exposure of rats to -15°C for four hours is followed by marked systolic function depression with no impairment of myocardial relaxation or passive elastic myocardial properties. In fact, although DPmax and developed pressure / volume relations were clearly depressed, no effects on relaxation constant and stress / strain relations were noted after cold stress. It was
interesting that, although developed pressure values were decreased at all levels of ventricular dilation in the hearts of stressed rats, no changes were observed in the slope of developed pressure / volume relations; that is to say, there were no modifications of the Frank-Starling mechanism. These results indicate that, even though contractile capacity was depressed at all levels of myofiber lengthening, profound cold stress did not affect length dependent activation, which bases the Frank-Starling mechanism.26-28) This seems to be a dysfunction consequent to impairment of maximum calcium-activated force rather than a decrease of myofilament calcium sensitivity.29) Aside from systolic function impairment, rat hearts of the P group disclosed ultrastructural features that documented the profound cold stress cardiac injury. Myofibrillar and mitochondrial intense disarrangements found in the myocardium of those rats confirmed that profound cold stress promotes intense muscle disturbances. The mechanisms through which whole body cold stress test triggers myocardial damage are to be more clearly defined. We are not aware of any specific publication that has assessed heart temperature in experiments of this kind, and we have not done that either. We understand that studies on whole body cold stress test are conducted without that concern because cardiac lesions are considered to be a consequence of the neurohumoral changes of the condition. Literature references support the assumptions that high plasma levels and intense catecholamine myocardial synthesis play a major role in myocardial lesions.5-8) The protection effect carried out by propranolol4) favors such a possibility.

There are still doubts as to whether CoQ10 administered orally is incorporated by myocardium. Some papers have reported that intracellular ubiquinone levels do not exhibit any changes when CoQ10 is administered exogenously,17,18,30,31) whereas others suggested CoQ10 incorporation.13,19,20,32) Indeed, there are reports of exogenous CoQ10 incorporation in to cellular membranes.32) Nevertheless, the number of papers referring to a CoQ10 myocardial protection effect when administered orally is expressive.13,17,18,20) Functional properties preservation is reported even without CoQ10 myocardial incorporation.17) It has been suggested that CoQ10 high plasma levels suffice for myocardial protection. In this respect, one study reported that CoQ10 plasma levels may double on the fourth day of exogenous administration.31) In any case, our structural data showed that 5-day oral administration of CoQ10 protects rodent myocardium against cold stress injury. Keeping in mind that increased adrenergic activity is the most frequent hormonal disarrangement observed in profound hypothermia, it can be assumed that the myocardial lesion occurs as a result of an oxygen supply / demand ratio unbalance. Assuming the final determining factor for cold stress induced myocardial lesion is an oxygen deficit, then it makes sense for CoQ10 to have acted protectively, since ubiquinone's benefits have been repeatedly proven
both for ischemia conditions\(^1,^{13-19}\) and oxidants action.\(^{21,22}\)

In addition, although clear ultrastructural protection was accomplished, it could be shown that exogenous CoQ\(_{10}\) does not protect systolic function from profound cold injury. Indeed, our data have shown that systolic function of hearts obtained from rats treated with exogenous administration of CoQ\(_{10}\) did not display any differences compared to non treated animals. Such discrepancy between the responses from structural and functional parameters to a cellular insult is not actually new. We can point out that the stunned myocardium example is the most widely known for the dissociation between structural data and functional variables against an injury. In stunned myocardium, systolic and diastolic function impairment can be observed concurrent to myocardial normal ultrastructure. In our case, it would not seem absurd that the structural lesion in the group treated with CoQ\(_{10}\) is the minimum lesion necessary to trigger the systolic dysfunction found in both groups. Also, the more severe lesion in the Placebo group, in addition to reaching the minimum, was not sufficient to make the dysfunction more severe.

Our data on ultrastructure lead us to the conclusion that CoQ\(_{10}\) exogenous administration protects the myocardial ultrastructure from injury resulting from profound hypothermia. The same efficiency could not be seen as preventive against functional changes.

**ACKNOWLEDGMENTS**

The authors thank Mr. Alrton Andrade Santos for his skillful technical assistance in the surgical preparation, Regína H. E. Alfarano, PhD for her editorial assistance and Fundação de Amparo a Pesquisa do Estado de São Paulo for the financial support (FAPESP 99 / 04533-4).

**REFERENCES**

28. Lakatta EG. Starling's law of the heart is explained by an intimate interaction of muscle length and myofilament calcium activation. J. Am. Coll. Cardiol. 1987; 10: 1157-64.


